Tissue Organ Distribution and Behavioral Effects of Platinum Following Acute and Repeated Exposure of the Mouse to Platinum Sulfate

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Platinum sulfate was administered intragastrically (IG) to adult male Swiss mice in a single dose at the 7 day LD_5 or LD_{25} level. Control groups received $0.25M$ H_2SO_4 (pH 0.85) or $0.14M$ NaCl. Open field behavior (ambulations, rearings) was measured, and tissue/organ Pt levels determined at 4 hr, or 1, 3, or 7 days post administration. At all times, the LD_{25} depressed ambulations significantly and rearings marginally. It did not effect exploratory ("hole-in-board") behavior. The LD_{25} resulted in disproportionately high tissue Pt levels relative to the LD_5. There were significant inverse correlations between behavior and tissue Pt levels for most tissues, but not for brain.

In related experiments, adult male mice were subjected to repeated IG administration of $Pt(SO_4)_2$ at the LD_1 level (one dose every 72 hr for up to 10 doses). Three days after administration of the final dose of each series, open-field and exploratory performance were measured and tissue/organ Pt levels determined. Tissue/organ Pt levels were variable but generally increased with dose number. No Pt was detected in the brain. Activity and explorations were marginally depressed. Only rearings correlated significantly with tissue Pt levels.

Introduction

Until relatively recently, human exposure to platinum (Pt) and Pt compounds has been limited mainly to the workplace (e.g., mining and refining and the manufacture and reprocessing of catalysts) and laboratory. However, accompanying the introduction of the automobile catalytic converter (1975 automobile model year), the therapeutic use of Pt complexes with antitumor activity (*1*), and the inevitable increase in coal utilization (which, overall,

will release significant quantities of Pt), a potential for widespread, long-term environmental dissemination and increased bioavailability of Pt exists.

Much of our knowledge of the biological effects of Pt has been derived from studies of Pt complexes with anti-neoplastic activity, primarily *cis*-dichloro-diamineplatinum(II) (*1, 2*). It has been observed that following parenteral administration, such complexes are rapidly excreted, primarily in the urine, mostly in unchanged form. In man and experimental animals, the half-time of elimination of these complexes is of the order of 1-15 hr for 80% of the dose, the remaining 20% being eliminated slowly over a period of weeks. The complexes tend to accumulate in the filtering organs (i.e., the kidney, liver, spleen, and thymus) and in populations of dividing cells. Thus, characteristic side effects of antitumor chemotherapy with Pt complexes are observed. Of great im-

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portance among these is necrosis of the proximal convoluted tubules (resulting from the accumulation of high levels of Pt in the kidney) which terminates in nephrosis. Destruction of the gastrointestinal epithelium also occurs and, in humans, is dose-limiting. In addition, high doses of cis-dichloro-diamineplatinum(II) are ototoxic, depress bone marrow function, and induce nausea and emesis (3-8).

Much remains to be learned about the interactions of other types of Pt compounds with biological systems. In animal studies, it has been observed that orally administered $PtCl_4$ is poorly absorbed (less than 0.5%) (9, 10). Of the absorbed Pt, between 50 and 70% Pt is excreted via the urinary route; the remainder via the fecal route (9-11). Via the intravenous route of administration, the highest levels of Pt are found in the kidney, spleen, and liver, and elimination half-times of the order of 2.5 to 7.5 days are observed (8-11). These retention/elimination characteristics appear to be similar to those of cis-dichlorodiamineplatinum(II) in humans (12).

Platinum compounds also have been shown to be allergenic in the majority of exposed industrial employees (13). Symptoms include bronchial asthma, allergic rhinitis, eczema, itching, and dermatitis (13, 14).

Except for cases of high sensitivity, it would appear that exposure to relatively high doses of Pt is required to elicit overt toxicity. However, it is of considerable importance to be cognizant of the fact that long-term, low-level (classically subpharmacological) exposure to environmentally available toxicants may result, in an insidious manner, in covert toxicity manifested, for example, by behavioral alteration (15, 16). In this context, nothing is known about Pt compounds.

Thus, employing the mouse as a model mammalian system, we undertook to investigate the effects of acute and repeated (subacute) exposure to Pt (sulfate) on: tissue/organ uptake of Pt, selected behaviors (open field, exploratory), and the relationship between tissue/organ Pt levels and behavioral effects.

Materials and Methods

Animals

Male random-bred Swiss mice (ICR strain, West Seneca Laboratories, West Seneca, NY) 6-8 weeks old, served as subjects in all studies. The animals were acclimated to the laboratory for at least one week prior to use. For all experiments except the dose determination study, they were housed individually in suspended metal mouse cages (Hoeltge, Inc., Cincinnati, Ohio). For the dose determination study, the animals were maintained in groups of five per double cage. All were fed ad libitum on Charles River RMH 1000 diet (Agway, Inc., Syracuse, NY), and were maintained on a 12 hr light/dark cycle at 22°C.

Reagents

All chemicals were of the highest grade available commercially.

Acute (Single-Dose) Exposure to Pt(SO₄)₂

Dose Determination. A 7 day mortality-dose response study was conducted for the intragastric (IG) route of administration. Lethal dose percentiles (LD_1. . .LD_{xx}) were calculated by probit analysis.

Tissue/Organ Uptake and Behavioral Effects. Subjects in this study were 160 animals.

The 7-day LD_5 and LD_{25} were selected for investigation. At these concentrations, the pH of the Pt(SO₄)₂ solutions was approximately 1.0. To compensate for possible effects of simultaneous exposure to low pH and sulfate, a low pH sulfate control (containing no Pt) was included in the study. The sulfate concentration and pH of this solution were identical to those of the LD_{25} dose. To identify possible effects on behavioral performance of exposure to low pH and sulfate, a saline control group also was investigated. Thus, the animals (groups of 40, randomly selected) received 144 mg Pt/kg (LD_5); 213 mg Pt/kg (LD_{25}); 0.25M sulfuric acid adjusted to pH 0.85 (pH of LD_{25} solution) with sodium hydroxide, or 0.14M sodium chloride. In each case, the volume administered was equivalent to 1% (on a volume/weight basis) of body weight. The animals were lightly etherized prior to administration for ease and consistency of dosing.

The animals were observed in an automated open field (model 1497, Lehigh Valley Electronics, Fogelsville, PA) at 4 hr or 1, 3 or 7 days post administration. Each subject was placed initially in the center circle (diameter = 15 cm) of the open field and was observed for 5 minutes. The dependent measures, which we view as providing an index of general activity, were total ambulations (automatically recorded) and total rearings (visually recorded). A rearing was scored each time the subject raised both forepaws off the floor in the absence of grooming behavior.

Immediately after observation, the animals were sacrificed by overdosing with ether and were dissected. Blood was obtained by cardiac puncture and the liver, spleen, kidneys, lungs, testes, skeletal muscle, cerebrum, cerebellum and medulla were removed, weighed and placed in acid washed test tubes.

In an extensive pilot study, it was established that the tissue Pt levels of animals not receiving Pt were below the detection limit of the assay. Therefore, only the tissues of Pt group animals were analyzed. Tissues of the Pt group animals were analyzed by flameless atomic absorption spectroscopy employing a wet ashing procedure modified that described by Tillery (17). Samples were collected in acid washed test tubes. Concentrated nitric acid, 5 ml, was added, and the samples were heated to 70°C to initiate digestion of the tissues. The nitric acid was boiled off and 5 ml concentrated HCl was added and boiled off; 2 ml of 70% perchloric acid was added and heated to approximately 200°C to complete digestion. The perchloric acid was boiled off, and a Pt-Sn complex was formed by the addition of a 2% stannous chloride solution in $3M$ HCl. The negatively charged complex was extracted with two 0.5 ml aliquots of 20 mM tri-n-octylamine in xylene. Extraction was necessary to obtain Pt free of interferring inorganic salts present in the tissues. Platinum levels were measured on a Perkin-Elmer Model 360 atomic absorption spectrophotometer equipped with a Model HGA-2100 graphite furnace. A 25 μl aliquot of the sample in xylene solution was placed in the graphite furnace. The sample was dried at 160°C, charred at 900°C, and atomized at 2700°C. Samples were compared to a standard curve included in each run, obtained from aqueous standards extracted into xylene in the same manner as the samples.

Analyses of variance (ANOVA) and overall association and correlation analyses were performed by using the Multivariance Computer Program, version 5.3 (18). Simple effects were investigated using Tukey's Honestly Significant Difference Test performed by the Statistical Package for the Social Sciences (SPSS) version 6.0, subprogram one way (19). Lethal dose percentiles (LD_{xx}) and confidence intervals were obtained by probit analysis (18) using an Isobol computer program (20).

Exploratory Behavior. Subjects were 160 male mice. Dosing was performed as described above in the Pt(SO$_4$)$_2$ single-dose open field study.

Behavioral observation was carried out by placing the subjects in a 5.7 cm wide circular runway formed from two concentric walls with an outside diameter of 34.3 cm. In the outside wall were four holes, 1.3 cm in diameter, centered 90° apart and 5.1 cm above the floor. Photocells were so arranged that each time the animal placed its snout into a hole the event was counted. Thus, the measure of exploratory behavior was the number of hole entries (automatically recorded) in a 5 min period. The runway was especially designed to minimize general activity and maximize exploratory activity.

Multiple Exposures to Subacute Doses of Pt(SO$_4$)$_2$

Open Field and Exploratory Behavior Studies. Subjects were 250 animals. Of these, 100 animals (plus an allowance for probable deaths) were assigned (randomly) both to the Pt and low pH groups, while 50 (plus an allowance) were assigned to the saline control group.

The experimental animals received 1 to 10 doses (IG) of Pt at the 7 day LD$_1$ level of 109 mg Pt/kg in a volume equivalent to 1% (v/w) of body weight. Low pH controls received an equal volume of 0.15M H$_2$SO$_4$ adjusted to pH 1.18 with NaOH (which was identical in sulfate concentration and pH to the Pt solution). Saline controls received an equivalent volume of 0.14M NaCl. Three days after administration, ten animals each from the Pt and low pH groups and five animals from the saline group were randomly selected and observed for behavioral alterations. The remaining animals were dosed again as described and this procedure was repeated for a total of ten dosings.

The animals were observed in the automated open field and the exploratory apparatus as in the single dose studies described above. Half of the animals in each treatment group were observed first in the open field and then in the exploratory apparatus. With the other half, the procedure was reversed.

Immediately following behavioral observation after doses 2, 4, 6, 8, and 10, the Pt group animals were sacrificed and a blood sample, the liver, kidneys, spleen, lung, gastrointestinal tract, testes, and brain were collected in acid-washed test tubes. Tissue Pt concentrations were determined as described above.

Results

Exposure to a Single Dose of Pt(SO$_4$)$_2$ at the LD$_5$ or LD$_{25}$ Level

Seven Day Mortality—Dose Response Studies (Dose Determination). The seven day IG LD$_{50}$ for Pt (SO$_4$)$_2$ [pH range = 1.06 (150 mg Pt/kg body weight) to 0.46 (500 mg Pt/kg)] was 280.5 mg Pt/kg (95% confidence limits: 237.6 - 320.1 mg Pt/kg). No deaths occurred in control groups.

Open Field Behavior and Tissue/Organ Distribution. The results of the open field study were analyzed by using a two-way (time of observation by treatment) multivariate ANOVA (Table 1). The time effect was significant overall and for ambulations, separately. The time effect for rearings approached significance. The treatment effect, which compares all subjects receiving each treatment with those re-

Table 1. Analysis of variance of open field behavior.

| Variable | Effects being tested | | |
	Time	Treatment	Time by treatment
Multivariate d.f.	6 and 286	6 and 286	18 and 286
F-Ratio	2.76 ($p<0.01$)	2.61 ($p<0.02$)	0.69 (N.S.)
Univariate d.f.	3 and 144	3 and 144	9 and 144
F-Ratios			
Ambulations	3.68 ($p<0.01$)	4.85 ($p<0.005$)	—
Rearings	2.38 ($p<0.07$)	2.43 ($p<0.07$)	—

ceiving other treatments regardless of the time of observation, also was significant overall and for ambulations, separately. Again, rearings approached significance. The time by treatment effect, which

tests for interactions of the two main effects (time and treatment) that are not simply additive, was not statistically significant.

To determine the basis of the time and treatment effects, the behavioral data (Fig. 1) were subjected to Tukey's Honestly Significant Difference Test (*19*). The time effect for ambulations was found to be due to the 4 hr animals having lower scores than the 1-day animals. However, neither of these groups differed significantly from the 3-or 7-day groups. The treatment effect for ambulations was due to the LD_{25} groups having lower scores than all other treatment groups (which did not differ among themselves). For rearings, the treatment effect only approached significance. However, the pattern for rearings paralleled that for ambulations.
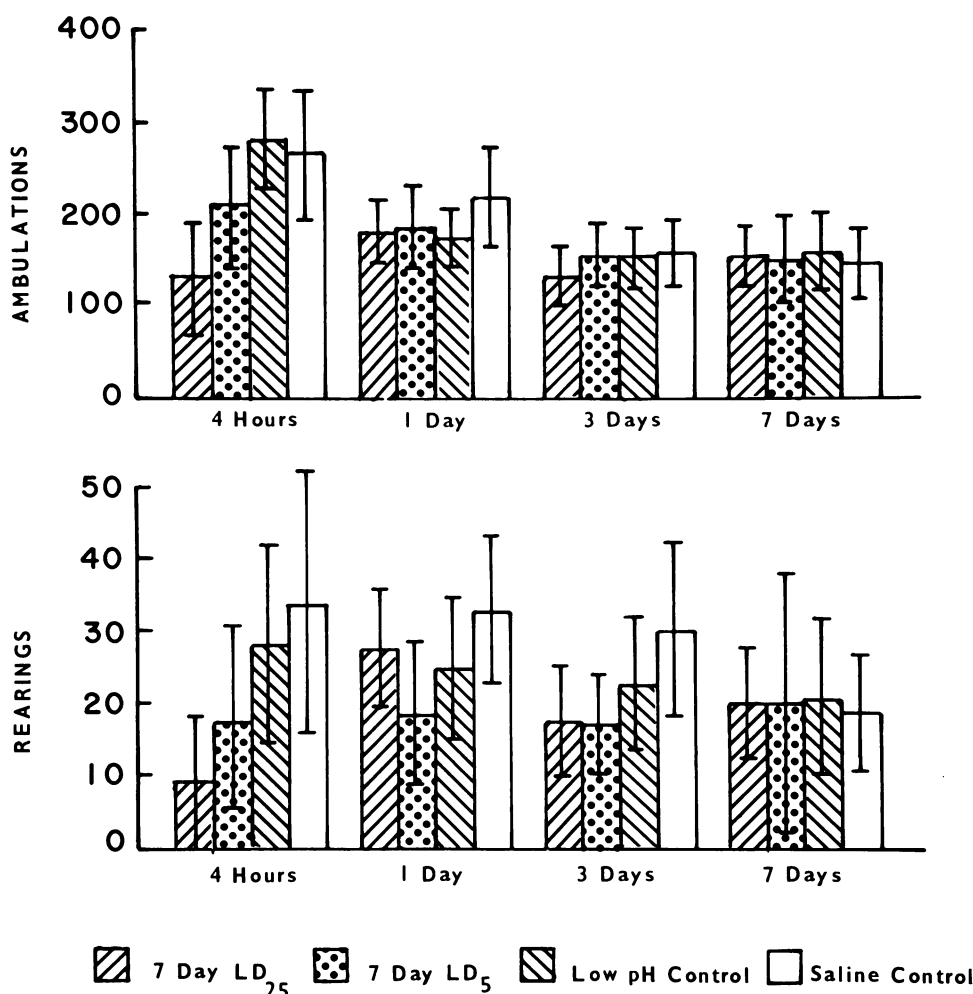
Tissue/organ Pt concentrations (for the Pt groups

FIGURE 1. Effects of $Pt(SO_4)_2$ on mouse open field behavior. $Pt(SO_4)$ was administered intragastrically to 6-8 week old male random bred Swiss mice in a volume equivalent to 1% (v/w) of body weight. LD_5 = 144 mg Pt/kg; LD_{25} = 213 mg Pt/kg; Low pH control = 0.25M H_2SO_4 adjusted to pH 8.5 (pH of LD_{25} solution) with NaOH; saline control = 0.14M NaCl.

Table 2. Analysis of variance of tissue/organ Pt concentrations in adult male mice receiving a single dose of Pt(SO₄)₂ at the LD₅ or LD₂₅ level.

Variable	Effects being tested		
	Time (post administration)	Dose	Time by dose
Multivariate d.f.	30 and 182.66	10 and 62	30 and 182.66
F-ratio	8.80[a]	9.19[a]	3.33[a]
Univariate d.f.	3 and 71	1 and 72	3 and 71
F-ratios			
Blood	3.80[b]	20.87[a]	1.85
Liver	2.71[c]	32.91[a]	1.03
Spleen	1.12	43.29[a]	0.79
Kidney	7.05[a]	13.24[a]	2.58
Lung	7.69[a]	20.31[a]	2.56
Testes	6.01[a]	77.75[a]	2.18
Muscle	8.04[a]	28.73[a]	5.09[b]
Cerebrum	12.71[a]	1.06	0.60
Cerebellum	45.61[a]	1.96	5.75[b]
Brain stem	12.03[a]	1.48	0.31

[a] $p < 0.001$.
[b] $p < 0.01$.
[c] $p < 0.05$.

only) were subjected to a multivariate ANOVA (Table 2). There was a significant multivariate time effect with significant univariate effects for all tissues except spleen. The multivariate dose effect also was significant with significant univariate effects for all tissues except brain. The multivariate time by dose interaction was significant; but only muscle and cerebellum had significant univariate effects.

The effects of time and dose on tissue/organ Pt concentrations are shown in Table 3. It is apparent that there is a time differential in the attainment of maximum Pt levels among different tissues/organs. Thus, Pt concentrations in the blood, kidney, lung, muscle, and brain tissue are highest at 4 hr post

Table 3. Tissue/organ Pt concentrations tissue at various times post administration in adult male mice receiving a single dose of Pt(SO₄)₂ at the LD₅ or LD₂₅ level.

Pt dose, mg/kg	Tissue	Pt concn, μg Pt/g wet weight[a]			
		4 hr	1 day	3 days	7 days
144	Blood	5.9 ± 4.3	3.4 ± 4.2	2.1 ± 0.9	1.2 ± 1.0
(LD₅)	Liver	8.1 ± 5.1	8.6 ± 12.1	4.9 ± 2.6	1.0 ± 1.3
	Spleen	2.2 ± 1.3	2.7 ± 3.2	2.4 ± 1.1	1.3 ± 0.5
	Kidney	12.8 ± 6.2	7.5 ± 7.2	6.3 ± 7.4	1.3 ± 1.3
	Lung	7.4 ± 8.3	2.6 ± 2.8	1.8 ± 0.5	1.4 ± 2.0
	Testes	1.3 ± 1.2	0.7 ± 0.7	0.5 ± 0.2	1.3 ± 1.0
	Muscle	0.6 ± 0.6	0.3 ± 0.3	0.2 ± 0.1	0.1 ± 0.1
	Cerebrum	0.9 ± 1.2	0.1 ± 0.2	0.0 ± 0.0	0.2 ± 0.1
	Cerebellum	0.9 ± 0.5	0.3 ± 0.3	0.0 ± 0.1	0.2 ± 0.2
	Medulla	0.6 ± 0.8	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
213	Blood	29.8 ± 31.6	15.9 ± 14.0	14.4 ± 12.8	6.5 ± 3.5
(LD₂₅)	Liver	28.5 ± 22.7	34.6 ± 33.0	37.6 ± 26.6	13.9 ± 7.9
	Spleen	12.7 ± 11.3	12.8 ± 11.2	18.7 ± 13.9	10.8 ± 5.9
	Kidney	48.5 ± 51.7	25.0 ± 17.7	20.2 ± 12.5	1.5 ± 0.7
	Lung	27.2 ± 18.5	16.4 ± 20.2	8.9 ± 6.8	3.8 ± 1.8
	Testes	4.3 ± 2.6	2.8 ± 2.1	3.1 ± 1.2	5.8 ± 2.2
	Muscle	3.7 ± 2.5	2.1 ± 2.4	1.0 ± 0.7	0.4 ± 0.2
	Cerebrum	1.4 ± 1.4	0.2 ± 0.4	0.1 ± 0.2	0.2 ± 0.1
	Cerebellum	1.6 ± 0.8	0.0 ± 0.0	0.1 ± 0.2	0.3 ± 0.2
	Medulla	0.8 ± 0.7	0.2 ± 0.4	0.0 ± 0.1	0.2 ± 0.1

[a] Means ± SD.

Table 4. Correlations between open field behavioral measures and tissue/organ Pt concentrations in animals receiving a single dose of Pt(SO₄)₂ at the LD₅ or LD₂₅ level.

Tests of the hypothesis of no association between behavior and tissue Pt concentrations	Overall $F = 2.45, p < 0.001$ d.f. = 20 and 134	
	Ambulations $F = 3.98,$ $p < 0.001$ d.f. = 10/68	Rearings $F = 3.35,$ $p < 0.001$ d.f. = 10/68
Correlation coefficients	d.f. = 78	
Blood	−0.487[a]	−0.394[a]
Liver	−0.452[a]	−0.346[a]
Spleen	−0.553[a]	−0.469[a]
Kidney	−0.384[a]	−0.271[b]
Lung	−0.413[a]	−0.345[a]
Testes	−0.476[a]	−0.390[a]
Muscle	−0.323[a]	−0.216
Cerebrum	−0.159	−0.097
Cerebellum	−0.211	−0.125
Brain stem	−0.195	−0.163

[a] $p < 0.01$.
[b] $p < 0.05$.

administration for both doses. Liver and spleen concentrations at the LD₂₅ level rise to a maximum at 3 days and decrease by 7 days. For the LD₅ level they peak at 1 day and then decrease. In the testes, the Pt concentration is high (relatively) initially, decreases one and three days post administration, and returns to the initial level or higher at seven days. Regarding dose, it is of interest to note that tissue/organ Pt concentrations in the LD₂₅ groups were three to ten times higher than in the LD₅ groups even though the high dose groups received only about 1.5 times as much Pt.

Relationship between Open Field Behavior and Tissue Pt Levels. The correlations between behavior and tissue/organ Pt concentrations are shown in Table 4. Again, the data for this analysis were derived from the Pt-treated animals only, since Pt concentrations were undetectable in control animals. The performance scores for individual animals for each behavioral task and the Pt levels of the tissues of each animal formed the basis of the

Table 5. Effects of exposure to multiple (repetitive) doses of Pt(SO₄)₂ at the LD₁ level on open field (ambulations and rearings) and exploratory behaviors of the mouse.

Behavior	Doses	Platinum (mean ± SD)	Low pH (mean ± SD)	Saline (mean ± SD)
Ambulations	1	172.0 ± 42.8	174.4 ± 18.6	160.0 ± 27.3
	2	193.6 ± 49.6	190.8 ± 28.7	174.6 ± 29.8
	3	181.2 ± 34.6	195.3 ± 49.9	208.2 ± 46.1
	4	190.0 ± 43.6	190.2 ± 35.3	175.6 ± 38.7
	5	171.5 ± 34.7	180.6 ± 44.9	184.0 ± 78.2
	6	196.1 ± 32.7	189.3 ± 5.96	207.2 ± 76.8
	7	134.9 ± 57.0	196.6 ± 58.7	186.2 ± 49.5
	8	165.9 ± 24.9	175.3 ± 39.7	187.2 ± 34.6
	9	141.4 ± 47.2	176.6 ± 34.1	184.0 ± 43.2
	10	176.7 ± 30.2	199.2 ± 53.3	200.8 ± 36.4
Rearings	1	40.1 ± 16.6	38.2 ± 19.9	29.4 ± 7.9
	2	42.3 ± 9.4	51.5 ± 15.2	36.8 ± 16.6
	3	40.7 ± 11.6	37.1 ± 8.9	35.8 ± 6.8
	4	42.9 ± 13.6	35.6 ± 12.7	31.6 ± 14.8
	5	37.8 ± 9.1	33.8 ± 8.0	39.2 ± 19.9
	6	33.6 ± 13.1	35.6 ± 18.1	31.6 ± 13.7
	7	25.2 ± 15.6	33.2 ± 12.6	45.0 ± 11.4
	8	25.6 ± 8.9	23.4 ± 10.0	34.2 ± 14.8
	9	21.6 ± 8.3	30.3 ± 9.3	27.8 ± 13.7
	10	23.3 ± 6.8	33.7 ± 13.9	39.6 ± 7.4
Explorations	1	29.4 ± 10.9	30.9 ± 10.8	36.6 ± 15.5
	2	28.7 ± 17.6	40.6 ± 12.2	42.2 ± 15.4
	3	37.6 ± 16.7	29.5 ± 18.7	33.4 ± 14.4
	4	38.2 ± 19.5	28.1 ± 14.1	20.0 ± 7.9
	5	27.2 ± 15.6	31.8 ± 20.2	36.2 ± 19.2
	6	27.1 ± 14.6	41.0 ± 13.3	43.4 ± 26.8
	7	28.8 ± 20.1	38.2 ± 10.5	40.0 ± 16.9
	8	35.5 ± 20.9	35.6 ± 13.1	34.6 ± 12.0
	9	30.1 ± 22.8	33.6 ± 17.1	34.8 ± 16.4
	10	17.9 ± 15.6	42.4 ± 14.1	47.4 ± 11.9

Table 6. Analysis of variance of open field and exploratory behavioral measures: Pt(SO₄)₂ multiple dose study[a]

| | Effects being tested | | |
Variable	Number of doses	Treatment	Doses × treatment
Multivariate d.f.	27/637.31	6/436	54/650.37
F-ratio	2.37 ($p<0.001$)	1.97 (N.S., $p<0.07$)	1.27 (N.S., $p<0.10$)
Univariate d.f.	9/220	2/220	18/220
F-ratios[b]			
Ambulations	1.61 (N.S.)	3.29 ($p<0.05$)	0.83 (N.S.)
Rearings	5.04 ($p<0.001$)	0.66 (N.S.)	1.69 ($p<0.05$)
Explorations	0.42 (N.S.)	3.86 ($p<0.05$)	1.49 (N.S., $p<0.10$)

[a]The data were analyzed by a two-way analysis of variance. The factors were: number of doses and treatment (platinum sulfate, low pH, saline).

[b]Univariate F-ratios normally are not examined if the corresponding multivariate F-ratio was not significant at the $p<0.05$ level. However, in these studies, while formally retaining the critical level of $p<0.05$, we are noting significance levels up to $p<0.10$.

analysis. The correlations were determined via multivariate tests of no association which indicate, in this case, whether the two sets of variables, behavior and tissue/organ Pt concentrations, were associated with one another. These tests revealed significant associations for both open field behavioral measures (ambulations, rearings) together and, also separately.

Ambulations were significantly correlated with all tissue Pt concentrations except those of brain. Likewise, rearings were correlated with all tissue concentrations except those of brain and skeletal muscle. Both ambulations and rearings had strong negative correlations with spleen, blood, testes, liver and lung (in decreasing order) and a weaker correlation with kidney. The negative correlations indicate that the more depressed behavioral scores were associated with the higher tissue/organ Pt concentrations.

Exploratory Behavior Study. These behavioral data were analyzed by using a two-way (time of observation by treatment) univariate ANOVA.

Significant effects of time ($F = 6.66$, d.f. = 3/144, $p < 0.001$) and time by treatment ($F = 1.92$, d.f. = 9/144, $p < 0.05$) were found. Tukey's Honestly Significant Difference Test of the time effect showed exploratory behavior higher at 4 hr than all other times with no difference among the other times.

The time by treatment interaction primarily appears to be due to the 4 hr low pH control group which explored significantly more than the 4 hr Pt treated or saline control groups. In fact, all groups were similar at 4 hr with the exception of the low pH group. The balance of the interaction is taken up by the fact that exploratory behavior decreased at 1, 3, and 7 days for all groups except the saline control group whose behavior remained constant across time.

Exposure to Multiple (Repetitive) Doses of Pt(SO₄)₂ at the LD₁ Level

Open Field and Exploratory Behavior. The means (± SD) for the three behavioral measures (ambulations, rearings and explorations) are presented in Table 5. Multivariate ANOVA of these data (Table 6) revealed a significant effect of dose number with rearings as the only significant univariate measure. The treatment effect approached significance ($p < 0.07$) with significant univariate F-ratios for ambulations and explorations. The doses by treatment interaction also approached significance with a significant univariate effect for rearings and with explorations approaching significance. Tukey's Honestly Significant Difference procedure revealed that rearings were depressed following doses 7, 8, 9, and 10 compared to dose 2 and were also depressed following doses 7 and 8 compared to dose 3. Tukey's procedure is not entirely efficient for unequal cell frequencies and failed to find any pairwise differences among treatments for ambulations and explorations despite significant overall F-ratios. Therefore, linear combinations of the treatments (Pt vs. low pH and saline, and low pH vs. saline) were tested as t-statistics to locate the effect. Ambulations and explorations were found to be depressed in the Pt groups relative to the control groups by this procedure while the control groups did not differ. The time by treatment interaction for rearings arose because, at times 7 and 10, scores for the Pt groups were lower than those for the saline controls. The time by treatment interaction for explorations arose because at time 10 only, the Pt group scores were lower than those of both control groups.

Tissue/Organ Pt Levels Following Exposure to Multiple (Repetitive) Doses of Pt(SO₄)₂ at the LD₁ Level. Tissue/organ Pt levels were analyzed by

Table 7. Tissue/organ Pt concentrations in adult male mice receiving multiple (repetitive) doses Pt(SO₄)₂ at the LD₁ level.

Number of doses	Tissue/organ Pt concns, μg/g (wet weight)[a]						
	Blood	Liver	Kidney	Spleen	Lung	GI tract	Testes
2	0.19 ± 0.11	0.15 ± 0.13	1.88 ± 2.12	0.23 ± 0.29	1.23 ± 3.27	0.13 ± 0.09	0.10 ± 0.12
4	0.50 ± 0.32	0.35 ± 0.26	2.71 ± 1.67	0.72 ± 0.43	1.40 ± 2.65	0.16 ± 0.12	0.28 ± 0.17
6	1.18 ± 0.63	0.58 ± 0.32	3.90 ± 1.78	1.95 ± 1.69	3.30 ± 7.78	1.04 ± 1.78	0.53 ± 0.34
8	1.07 ± 1.05	1.22 ± 1.33	6.84 ± 4.86	2.61 ± 3.69	4.51 ± 5.56	0.29 ± 0.27	0.60 ± 0.35
10	1.03 ± 0.77	0.96 ± 0.53	8.29 ± 8.48	2.11 ± 1.70	8.30 ± 8.91	0.45 ± 0.50	0.57 ± 0.36

[a]Means ± SD.

using a one-way (dose) multivariate ANOVA.

The tissue Pt concentrations are presented in Table 7. Brain levels were uniformly undetectable (less than 0.15 μg Pt/g tissue) and, therefore, were not recorded. Multivariate ANOVA revealed a significant effect of dose number ($F = 2.14$, d.f. = 28/142, $p < 0.01$). The dose effect also was significant for blood ($F = 4.09$, d.f. = 4/45, $p < 0.01$), liver ($F = 4.29$, d.f. = 7/45, $p < 0.01$), kidney ($F = 3.55$, d.f. = 4/45, $p < 0.05$) and testes ($F = 5.80$, d.f. = 4/45, $p < 0.001$), and very nearly significant for spleen ($F = 2.56$, d.f. = 4/45, $p < 0.0511$). In all cases, the effect was one of higher Pt concentrations at higher numbers of doses.

Relationship between Behavioral Measures (Open Field and Exploratory) and Tissue/Organ Pt Levels. The results of the overall tests of association and the correlations between behavioral measures and tissue Pt levels (for the Pt-treated groups observed after 2, 4, 6, 8, and 10 doses) are presented in Table 8.

A significant overall association was found. However, the only individual behavioral measure significantly associated with tissue Pt levels was rearings. Significant inverse correlations were found between rearings and liver, testes, kidney, spleen, and blood Pt levels.

Discussion

In 1975 Moore et al. (*10*) reported on the biological fate of single doses of [191]PtCl₄ in the rat. They observed that most of the perorally administered Pt was not absorbed; being eliminated in the feces. In the studies we report, exposure to a single IG dose of Pt(SO₄)₂ at the LD₅ or LD₂₅ levels resulted in systemic distribution of a quantity of Pt representing only a small fraction of the administered dose. Thus, although we did not analyze feces, and cannot, therefore, confirm their findings directly, our findings are consistent with those of Moore et al. (*10*).

Tissue Pt levels following exposure to a single LD₂₅ dose of Pt(SO₄)₂ are disproportionately high relative to the tissue levels following exposure to a single dose at the LD₅ level. We have no explanation for this observation. However, it is conceivable that

Table 8. Relationship between open field and exploratory behavioral measures and tissue Pt levels: Pt(SO₄)₂ multiple (repetitive) exposure study.[a]

Tests of no association between behavioral and tissue measures	Overall F = 1.683, d.f. = 21/115.4085, $p < 0.05$		
	Ambulations F=1.24, df=7/42, N.S.	Rearings F=2.80, df=7/42, $p < 0.02$	Explorations F=1.47, df=7/42, N.S.
Correlation coefficients (d.f. = 49)			
Blood	−0.132	−0.285[b]	−0.088
Liver	−0.210	−0.425[c]	−0.112
Kidney	−0.259	−0.371[c]	−0.237
Spleen	−0.194	−0.301[b]	−0.035
Lung	−0.231	−0.232	−0.357[c]
GI	0.010	−0.025	−0.044
Testes	−0.228	−0.378[c]	−0.047

[a]Tissue Pt levels were determined only in the Pt dosed animal groups. In all other groups, tissue/organ levels were below the limit of detectability.
[b]$p < 0.05$.
[c]$p < 0.01$.

the higher Pt dose may damage or stress the GI tract in some manner, resulting in increased Pt absorption. Alternatively, or in addition, the higher Pt concentrations found in the blood and kidneys of animals dosed at the higher level may damage the kidneys [which normally appear to eliminate Pt quite efficiently (21, 22)], resulting in decreased Pt excretion. Furthermore, it is well known that some metals, such as cadmium and lead, bind to proteins and in some cases (e.g., Cd) induce the synthesis of their own binding proteins (23, 24). Platinum may function similarly.

Acute exposure to $Pt(SO_4)_2$ significantly depressed ambulations in the open field and marginally depressed rearings (but only at the LD_{25} dose level). For ambulations, this pattern persisted from 4 hr through 7 days after administration, although the effect was most pronounced at 4 hr (Fig. 1). A correlation analysis (Table 4) revealed highly significant negative correlations between open field behaviors and tissue levels of Pt for all tissues except brain (and, in the case of rearings, skeletal muscle). Since brain Pt levels do not appear to be related to behavior, it is not clear how tissue Pt levels affect behavior. Waters et al. (25), working with alveolar macrophages in tissue culture, reported a 50% loss of viability (ability to exclude Trypan Blue) after a 20 hr exposure to $300mM$ $PtCl_4$. Protein and RNA synthesis were inhibited by 50% by $60mM$ Pt and DNA synthesis was inhibited by 50% by $10mM$ Pt. The maximum tissue Pt concentration observed in our study was approximately 50 ppm found in the kidney 4 hr post administration of a single LD_{25} dose of $Pt(SO_4)_2$. A level of 50 ppm is approximately equivalent to $250mM$ Pt. Therefore, by analogy to the findings of Waters et al. (25), cellular processes may have been disrupted to some extent in the LD_{25}-dosed animals which could have produced general malaise and depressed behavior.

Exposure to a single dose of $Pt(SO_4)_2$, even at the LD_{25} level, did not appear to alter "hole-in-board" exploratory behavior of the mouse. While there were significant effects of time and a time by treatment interaction, scrutiny of these effects indicates that they were not related to Pt exposure per se. The high rate of explorations of the 4 hr low pH control group (see Results), for which no satisfactory explanation is presently available, probably sufficiently accentuated the difference between the 4 hr time and all other times to account for the significant main effect of time as well as the significant time by treatment interaction.

Chronic exposure to Pt was simulated by the use of a limited multiple dose experimental design (1 to 10 doses). Each dose equaled the 7 day LD_1, which is approximately 40% of the 7 day LD_{50}. Thus, total exposure, following 10 doses, was substantially greater than the 7 day LD_{50}. In this study, tissue Pt levels: were found to be low across doses, were highly variable among tissues, and generally increased with dose number, except for the brain in which no Pt was detected (Table 7). The absence of Pt in the brain is not surprising since the highly charged Pt cation should be excluded by the blood-brain barrier.

Analysis of variance of the open field and exploratory behavioral measures obtained in the multiple dose study indicated a significant multivariate effect of dose. The multivariate effects of treatment and the doses by treatment interaction approached significance, and a number of the univariate F-ratios were significant (Table 6). Relative to dose, there was a highly significant difference in rearings between control and experimental animals. Also, rearings showed significant negative correlations with tissue Pt levels (Table 8). In light of the very low tissue/organ Pt levels (Table 7), these observations would appear to be highly significant.

A comparison of the single and multiple dose studies indicates a consistent relationship between the tissue/organ Pt levels and behavior (particularly rearings). In the single dose study, relatively high tissue Pt levels were associated with strongly significant behavioral effects while in the multiple dose study, relatively low tissue Pt levels were associated with weak behavioral effects. In both cases, the correlations between tissue Pt levels and rearings were generally significant, with the notable exception of the brain. Since these correlations involved a number of tissues, but not the brain, a general systemic effect, rather than a neurotoxic effect, seems to be indicated. Pt compounds have been shown to inhibit DNA, RNA, and protein synthesis in vitro (25) and in vivo (1, 21). Our findings suggest that Pt has a depressing effect on cellular processes and exposure to Pt above a threshold level results in general malaise which is manifested as a depression of certain behaviors. As Pt levels increase, the interference with cellular processes and, therefore, behavioral depression is likewise increased.

In summary, $Pt(SO_4)_2$ administered via the IG route appears to be poorly absorbed. However, with the exception of brain tissue, the absorbed Pt achieves general systematic distribution. With repeated exposure, tissue concentrations tend to increase with dose; again, with the exception of brain which did not accumulate Pt. While Pt did affect behavior under conditions of single and multiple dose exposure, these effects were weak except when very high (LD_{25}) individual doses were employed. In the latter case, the behavioral effects were more pronounced and lasted for up to 7 days post admin-

istration (end of observation). The consistent pattern of relationship between tissue levels of Pt and behavior is suggestive of interference with cellular processes induced by Pt at concentrations greater than some threshold value. This interference is manifested as behavioral malaise.

It is difficult to predict the potential danger of increased anthropogenic redistribution of Pt in the environment. Toxicity has been observed in humans exposed to high Pt levels in the workplace or during antitumor chemotherapy. However, such dose levels are many times those that would ever be expected to occur in the general environment. Nevertheless, since exposure to Pt appears to alter certain behavior of the mouse, the possibility of subtle adverse biological and behavioral effects resulting from long-term low level (environmental) exposure of humans cannot be disregarded especially in the light of the fact that Pt can be methylated (26) in a manner analogous to that of mercury (27). By altering its physicochemical properties, methylation may, as in the case of mercury (28) greatly enhance the toxicity of Pt and its bioaccumulatability.

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